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HLA Polymorphism in the Havasupai: Evidence for Balancing Selection

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Summary

The characterization and analysis of genetic variation at the HLA loci provides important insight for population geneticists trying to understand the evolutionary forces that have shaped human populations. This study describes the HLA-A and HLA-B loci serotyping and statistical analysis on an isolated Native American population, the Havasupai of Arizona. Four alleles at the HLA-A locus were identified, while eight alleles were found at the HLA-B locus. These variants were present as 20 of 32 potential two-locus haplotypes, with five of the six most common haplotypes exhibiting high positive linkage disequilibrium. Significant homozygote deficiency (heterozygosity excess) was detected both at HLA-A and at HLA-B. This deviation from Hardy-Weinberg proportions was not attributable to nonselective causes such as different allele frequencies in males and females or avoidance of consanguineous matings. In addition, the distribution of alleles at both HLA-A and HLA-B was more even than expected from neutrality theory; that is, the observed Hardy-Weinberg homozygosity was only 62.4% of that expected under neutrality. These observations suggest that balancing selection is of major importance in maintaining genetic variation at HLA-A and HLA-B.

Introduction

The genes of the HLA complex (the major histocompatibility complex, MHC, in humans) are among the most polymorphic known in humans (e.g., see Baur et al. 1984; Hedrick et al. 1991*a*, 1991*b*). Because a number of these tightly linked genes have multiple alleles, the various combinations of these alleles potentially give rise to an extremely large number of haplotypes. Human populations studied for HLA variation generally show substantial differences in allele and haplotype frequencies (e.g., see Baur et al. 1984). Further, various HLA alleles show significant and specific associations with a number of diseases, especially those of an autoimmune origin (e.g., see Tiwari and Terasaki 1985; Thomson 1988). This genetic and population information (and the availability of data because of the medical implications of HLA) make the HLA system attractive to population geneticists interested in investigating the action of evolutionary forces, such as natural selection, genetic drift, and gene flow, in human populations.

In many studies of HLA variation, haplotype frequencies and linkage disequilibria are estimated from genotypic frequencies because family data are not available. Populations in which haplotypes can be accurately deduced from family data and for which other population parameters, such as the level of inbreeding, population size, amounts of admixture and fertility, are available provide an important opportunity to address population-genetic issues. For example, the Hutterites have been extensively examined, and there has been great success in understanding some of the evolutionary factors that are important in influencing HLA variation in this closed population (e.g., see Morgan et al. 1980, 1986; Kostyu et al. 1989; Ober et al. 1992). One population indigenous to North America, the Havasupai of Arizona, is particularly well suited to population-genetic studies because, as in the Hutterites, much of this population information is available.

The Havasupai are a small ($N = 594$) tribe of Yuman-speaking people for whom complete pedigrees are available dating back to the late 1800s. The average inbreeding coefficient among the Havasupai has been conservatively estimated to be 1%–2% (Markow and

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Martin, in press). The Havasupai live in a quite remote site, a side canyon of the Grand Canyon, and their village, Supai, is not accessible by any road. Their isolated location becomes advantageous in population studies, in that the nature and extent of the group's interaction with other peoples is quite limited and easily documented.

In the present study, we characterize the variation present in the Havasupai for the HLA-A and HLA-B loci and examine the evidence, from a population survey, for selection maintaining genetic variation in this system. We present data for 122 Havasupai having no recorded ancestors from outside the tribe since the first census in 1897.

Material and Methods

Tissue Typing

During 1990 and 1991, blood samples were obtained from 122 Havasupais. Samples from Supai village were taken by helicopter to Hualapai Hilltop, from where they were driven to Arizona State University, logged in, and driven to the histocompatibility laboratory at GENETRIX for testing. Samples were always transported at room temperature. At certain times, it was not feasible to type fresh blood. In those cases, typing was performed on cells from cell lines created by Epstein-Barr virus (EBV) transformation at Arizona State University. HLA-A typing and HLA-B typing were carried out on either peripheral blood lymphocytes or EBV-transformed cell lines by using the standard NIH microlymphocytotoxicity technique (Terasaki et al. 1978), with both commercial typing trays and local reagents.

Allele and Genotype Frequency Analysis

We used the gene-counting method of determining allele and genotype frequencies. We first compared the observed allele-frequency distribution with that expected under the neutrality model when there are the same number of alleles (k) and the same observed sample number of gametes ($2N$). This test (Watterson 1978) is based on the sampling theory developed by Ewens (1972) and can be carried out by using the computer algorithm of Stewart (1977).

Next, we compared the observed numbers of homozygous and heterozygous genotypes to that expected on the basis of the allele-frequency estimates. To determine significance, first we calculated the expected frequency of homozygotes for a locus, $\sum p_i^2$, where p_i is the estimated frequency of the ith allele. We then calculated the probability

$$P = \binom{N_{ho}}{N}(1-\sum p_i^2)^{N-N_{ho}}(\sum p_i^2)^{N_{ho}}, \qquad (1)$$

where N_{ho} is the number of homozygotes in a sample of N individuals. We did this for all N_{ho} values less than or equal to that observed in the sample and summed these probabilities. In most other studies of variation of HLA, the genotypic frequencies are consistent with Hardy-Weinberg proportions (e.g., see Morgan et al. 1980; Baur et al. 1984). However, in a few instances a deficiency of homozygotes (excess of heterozygotes) has been observed (e.g., see Degos et al. 1974; Black and Salzano 1981). In other words, in this part of the analysis we have used a one-sided test, a strategy that does have appeal for exact tests (Weir 1990), particularly when there are multiple alleles.

In order to determine whether different allele frequencies in the males and females may have influenced genotypic frequencies, we separately tabulated allele frequency by sex and then calculated the observed frequency of homozygous genotypes that is due to this effect, as

$$P_{ii} = \sum p_{im}p_{if}, \qquad (2)$$

where p_{im} and p_{if} indicate allele frequencies in males and females, respectively, for the ith allele.

Another factor that can influence genotypic frequencies is the avoidance of consanguineous matings in a finite population (Jacquard 1974; Thompson and Roberts 1980). The potential effect of this factor when both full-sib matings and first-cousin matings are excluded and discrete generations are assumed can be estimated from the approximate formula

$$f = -\frac{1}{N_e - 10}, \qquad (3)$$

where f is the inbreeding coefficient and N_e is the effective population size (Jaquard 1974). N_e can be estimated here as

$$N_e = \frac{4N_fN_m}{N_f + N_f}, \qquad (4)$$

where N_f and N_m are, respectively, the number of females and the number of males in the parental generation (Thompson and Roberts 1980).

Haplotype Frequency Analysis

Haplotypes could be directly determined from family data in 119 of the 122 people tested. Linkage (game-

tic) disequilibrium can be measured for haplotype A_iB_j as

$$D_{ij} = x_{ij} - p_i q_j , \quad (5)$$

where x_{ij} is the observed frequency of the haplotype, p_i is the frequency of allele A_i, and q_j is the frequency of allele B_j. The significance level of the disequilibrium values was determined by using exact probabilities, i.e., determining the exact probability of obtaining an absolute linkage disequilibrium value greater than or equal to that observed. To calculate this value, the probability of given numbers of gametes A_iB_j, $A_i\bar{B}_j$, $\bar{A}_iB_j$, and $\bar{A}_i\bar{B}_j$ (where the overhead rule indicates "not"), conditional on the numbers of alleles at the two loci, N_{A_i} and N_{B_j}, is calculated as

$$P(N_{A_iB_j}, N_{A_i\bar{B}_j}, N_{\bar{A}_iB_j}, N_{\bar{A}_i\bar{B}_j} \mid N_{A_i}, N_{B_j}) = \frac{N_{A_i}!N_{\bar{A}_i}!N_{B_j}!N_{\bar{B}_j}!}{N_{A_iB_j}!N_{A_i\bar{B}_j}!N_{\bar{A}_iB_j}!N_{\bar{A}_i\bar{B}_j}!(2N)!} , \quad (6)$$

where $2N$ is the total number of gametes (after Weir 1990). The number of A_iB_j gametes may vary from 0 to the lesser of N_{A_i} and N_{B_j}. For each of these values of $N_{A_iB_j}$, we checked to determine whether the absolute linkage disequilibrium value, $|D_{ij}|$, is greater than or equal to the observed value. If so, the probability is calculated from equation (6) and added to the sum of such probabilities. The same process is carried out for all possible A_iB_j combinations.

To determine which probability values are significant, a sequential Bonferroni test for multiple values was used (Rice 1989). The calculated probability values for different haplotypes were ordered and then compared to determine whether they were smaller than $\alpha/(1 + m - i)$, where α is the significance level, m is the number of probability values (two-locus gametes), and i is the rank of the probability value. Note that this is a more appropriate (and more conservative) way to test for significance than is the traditional approach of assuming that each haplotype can be tested independently.

The extent of linkage disequilibrium overall can be calculated by using several different measures (Hedrick and Thomson 1986; Hedrick 1987). We will calculate the total extent of association, using

$$D^* = \frac{D^2}{H_A H_B} , \quad (7)$$

where $D^2 = \sum_{i=1}^{k} \sum_{j=1}^{l} D_{ij}^2$, $H_A = 1 - \sum_{i=1}^{k} p_i^2$, and $H_B = 1 - \sum_{j=1}^{k} q_j^2$.

Table 1

Allele Frequencies for HLA-A and HLA-B among the Havasupai (*N* = 122)

Allele	Frequency
HLA-A:	
A2	.537
A24	.189
A30	.004
A31	.270
HLA-B:	
B5v	.119
B27	.037
B35	.164
B39	.061
B48	.422
B51	.086
B60	.102
B61	.008

Results

Four alleles were detected for the HLA-A locus, three of which occurred at substantial frequencies (table 1). The most common allele was A2, with a frequency of .537, while the rarest allele was A30, for which only one copy in the total of 244 gametes was observed. The HLA-B locus showed greater polymorphism, with eight alleles being present and with the most common being B48. Two splits of B40—B60 and B61—were also identified, but B61 was at low frequency, with only two copies present. Two specificities for B5 were identified—B51 and what we will refer to as "B5v" to indicate an unusual B5 that is not B52. All of these alleles have been checked for their amino acid sequence (except for B39, which is now being sequenced). They appear to be homogeneous within HLA serotype (allele) for their amino acid sequence, and all sequences are known from other populations (P. W. Hedrick, unpublished data).

Twenty haplotypes of the possible 32 were found (table 2), the most common being A2 B48, with a frequency of .290. Five other haplotypes—A2 B5v, A2 B51, A31 B35, A31 B48, and A24 B60—occurred at substantial frequencies (.084–.122). Table 2 also presents the extent to which these haplotypes exhibit linkage disequilibrium. Six haplotypes—A2 B5v, A2 B48, A2 B51, A24 B39, A24 B60, and A31 B35—showed significant positive disequilibria; five of these were among the six most common haplotypes. In total, these five haplotypes constitute 175 (73.5%) of the total 238

Table 2

Linkage Disequilibria and Exact Probabilities for 238 HLA-A and HLA-B Locus Haplotypes

A	B	Observed	Expected	D_{ij}	P
A2	B5v	27	15.18	.050***	.000
	B27	3	.88	.008	.308
	B35	5	21.14	−.068***	.000
	B39	3	8.13	−.022	.007
	B48	69	54.20	.062**	.000
	B51	20	11.38	.036***	.000
	B60	1	13.01	−.050***	.000
	B61	1	1.08	−.000	1.000
A24	B5v	0	5.29	−.022	.008
	B27	2	1.70	.001	1.000
	B35	8	7.37	.003	.824
	B39	12	2.84	.038***	.000
	B48	2	18.91	−.071***	.000
	B51	0	3.97	−.017	.035
	B60	21	4.54	.069***	.000
	B61	0	.38	−.002	1.000
A30	B5v	0	.12	−.000	1.000
	B27	0	.04	−.000	1.000
	B35	0	.16	−.001	1.000
	B39	0	.06	−.000	1.000
	B48	0	.42	−.002	1.000
	B51	0	.09	−.000	1.000
	B60	0	.10	−.000	1.000
	B61	1	.01	.004	.008
A31	B5v	1	7.41	−.027	.005
	B27	4	2.38	.007	.250
	B35	26	10.32	.066***	.000
	B39	0	3.97	−.017	.029
	B48	29	26.47	.011	.461
	B51	1	5.56	−.019	.017
	B60	2	6.35	−.018	.047
	B61	0	.53	−.002	.609

** $P < .01$.
*** $P < .001$.

haplotypes observed. Three haplotypes—A2 B35, A2 B60, and A24 B48—were observed in significantly lower numbers than expected. In other words, even though we used a conservative approach to test disequilibrium, the number of haplotypes to have significant disequilibrium was quite high—9 of 32, or 28.1% (see Discussion).

We also counted the number of individuals who are homozygous for each allele and examined whether these numbers correspond to the number expected on the basis of Hardy-Weinberg proportions. We categorized the population into four classes to determine whether, in the different groups—female or male, or born before 1954 or in 1954 or later—there is any structure, by birth cohort or gender, for heterozygote or homozygote number. From table 3, it is clear that there is no structure by birth cohort or gender, for HLA-A ($\chi^2 = 1.84$, df = 3) or for HLA-B ($\chi^2 = 2.81$, df = 3). Of course any structure would be expected to result in a reduction in the observed heterozygosity (an increase in the observed homozygosity), because of the Wahlund effect (e.g., see Hedrick 1985). For both loci, there were significantly fewer homozygotes overall than expected by chance when exact probabilities are used; that is, the probability of obtaining, by chance, 38 or fewer homozygotes at HLA-A was $P = .0317$, and the probability of obtaining 21 or fewer homozygotes at HLA-B was $P = .0405$.

Using the observed frequencies of homozygotes at the two loci (.312 at HLA-A and .172 at HLA-B), we calculated the expected number of two-locus homozygotes, both types of single-locus homozygotes, and double heterozygotes and compared them with the observed numbers (double heterozygotes, observed = 69, expected = 69.54; homozygous for HLA-A and heterozygous for HLA-B, observed = 32, expected = 31.46;

Table 3

Observed and Expected Numbers of Heterozygotes and Homozygotes for Individuals Born Before 1954 and Those Born in 1954 or Later, for Males and Females

	Heterozygotes	Homozygotes
HLA-A:		
Born before 1954:		
Females	22	8
Males	22	7
Born 1954 or later:		
Females	22	12
Males	18	11
Total:		
Observed	84	38[a]
Expected	73.57	48.43[a]
HLA-B:		
Born before 1954:		
Females	25	5
Males	22	7
Born 1954 or later:		
Females	31	3
Males	23	6
Total:		
Observed	101	21[b]
Expected	92.44	29.56[b]

[a] $P = .0317$.
[b] $P = .0405$.

heterozygous for HLA-A and homozygous for HLA-B, observed = 15, expected = 14.46; and double homozygotes, observed = 6, expected = 6.38). Obviously the observed and expected values are close in all categories, and, for these data, $\chi^2 = 0.08$, a nonsignificant value with df = 1, indicating no two-locus association at this general level.

One factor that can cause an increase in heterozygosity over Hardy-Weinberg expectations is different allele frequencies in females and males (Robertson 1965; Purser 1966; Hedrick 1990). Expression (2) was used to calculate the observed frequency of heterozygotes, and the expected heterozygous proportion was calculated by using the mean allele frequency. When this approach and the observed male and female frequencies are used, the relative percentage increase in heterozygosity, versus that expected for HLA-A, is only 0.33%, and that for HLA-B is only 0.65% (this was calculated by using the heterozygosities as (observed − expected)/expected). In other words, the overall influence of different male and female frequencies on heterozygosity appears to be minor. We also examined this effect in the two cohorts as described above. For HLA-A, the value was low for both cohorts, an increase of 0.15% and 0.10% for the early and late cohorts, respectively. For HLA-B, much of the effect, still far below the total amount that we observed, was concentrated in the early cohort, with an increase of 2.66% as compared with an increase of 0.34% in the late cohort.

Another factor that can increase heterozygosity is the avoidance of close inbreeding in a finite population. In fact, there are no known first- or second-degree matings in the Havasupai. One first-cousin mating is known, but the partners in this case were not aware of their relationship before their marriage, suggesting that first-cousin matings are also avoided. With expression (4) and the numbers of females and males born in the two most recent 20-year periods in which we know survival to age 20 years—1928–1947 and 1948–1967—then N_e is estimated to be 98.9 and 214.8, respectively. As a result, then f from expression (3) is −.0112 and −.0049 for these two periods, respectively, resulting in a potential increase of heterozygosity of only 1.12% and 0.49%, respectively. In other words, when the approximation given in expression (3) is used, avoidance of close inbreeding would appear to have a negligible effect on genotypic frequencies.

We also compared the observed distribution of allele frequencies with that expected under neutrality, using the Ewens-Watterson test (table 4). In this case, the Hardy-Weinberg homozygosity ($\sum p_i^2$) was much less than that expected under neutrality, for both HLA-A and HLA-B, although the probabilities in both cases were not statistically significant ($P = .132$ for HLA-A, and $P = .123$ for HLA-B). For HLA-A, the observed homozygosity was only 64.4% of that expected under neutrality, while for HLA-B it was only 62.4% of that expected under neutrality. These results imply that the allelic frequency distribution tends to be more even than predicted under neutrality.

The calculated value of D^*, the overall measure of disequilibrium we used, was .0738 in this sample. From table 1 of a paper by Hedrick and Thomson (1986), the most appropriate combination of parameters for a comparable expected value under neutrality is a sample size of 200 haplotypes and six alleles at each locus. In this case, the value of D^* for $4N_ec = 10$ is .068, quite close to that observed. This value of $4N_ec$ is similar to that which Hedrick and Thomson (1986) found for HLA-A and HLA-B in several other groups. Because we know that $c = .008$ between HLA-A and HLA-B, for a combination of genetic drift and mutation to generate this much disequilibrium, then the long-term N_e would need to be around 312. For the Havasupai, this number is actually not far from the suggested effective population size (see Discussion).

However, one major difference between the observed disequilibrium and the neutrality expectations is that, for the Havasupai, six of the seven most common haplotypes had high positive normalized disequilibrium, i.e., D' values (Lewontin 1964; Hedrick and Thomson 1986). For example, haplotypes A2 B5, A2 B51, and A24 B60 had D' values of .922, .896, and .846, respectively. This large proportion of high positive D' values was not seen in the neutrality predictions of Hedrick and Thomson (1986).

Discussion

HLA data from the Havasupai show strong evidence of some type of balancing selection, from three different analytical approaches. First, the frequencies of homozygotes at both HLA-A and HLA-B are significantly less than expected on the basis of allele frequencies. This excess of heterozygotes does not appear to result from nonselective factors, such as different male and female allele frequencies or avoidance of consanguinity, that may increase the frequency of heterozygotes. There did not appear to be significant structure in the sample for heterozygosity, when examined either by sex or by generation.

Homozygote deficiencies for the HLA system have also been reported previously for several other human

Table 4

Expected and Observed Hardy-Weinberg Homozygosity for HLA-A and HLA-B

	No. of Alleles (k)	Sample Size ($2N$)	Hardy-Weinberg Homozygosity (f)		
			Expected	Observed	P
HLA-A	4	244	.616	.397	.132
HLA-B	8	244	.388	.242	.123

populations (e.g., see Degos et al. 1974; Black and Salzano 1981), ones that also have a limited number of alleles. Although we do not know the exact basis of the finding that populations with low numbers of alleles seem to be the ones that also show homozygote deficiencies, there are several possible reasons. For example, the possibility of selection between homozygotes and heterozygotes is more likely when the frequencies of the two classes are more equal. In many populations, the frequency of HLA heterozygotes is much greater than 90%, thereby reducing the opportunity for selection. Another possibility is that isolated populations may in fact be subjected to selective factors that are not present in other societies. For example, health care for infectious diseases may now be—or, in the recent past, may have been—less thorough for such isolated groups. Finally, in populations of low HLA variation there may be less heterogeneity within serotypic class than in more diverse populations. For example, in the Havasupai neither HLA-A nor HLA-B appears to have any amino acid variation within serotype (P. W. Hedrick, unpublished data), while many Caucasians have substantial variation. If these variants have different susceptibility to pathogens, then the heterozygosity from the pathogens's perspective may be even larger than expected on the basis of the serotype homozygosity.

Second, the distribution of alleles at both HLA-A and HLA-B tends to be more even than expected under neutrality. The observed homozygosity over the two loci was only 63.4% of that expected under neutrality. Again, this type of result has been observed for HLA loci in samples from a diversity of other populations (Hedrick 1983; Hedrick and Thomson 1983; Klitz et al. 1986), including Native Americans (Hedrick 1983). Unlike these other populations, our Havasupai sample represents a large proportion (one-third) of the total adult population, suggesting that the observed allele frequencies in this sample are probably quite close to the actual population frequencies. We should note that the Havasupai population is probably not at neutrality equilibrium (nor are most other human populations). However, we still feel that neutrality measures are useful both in understanding the pattern of genetic variation in this population and in comparing it with other populations.

Finally, the number of haplotypes showing significant linkage disequilibrium was quite high even though we used a more conservative test than is ordinarily used for HLA data. In particular, five of the six common haplotypes were much more frequent than expected, suggesting that there has been selection favoring these two-locus combinations. The relative evenness in frequency of the common haplotypes is also suggestive of balancing selection. However, as shown above, general measures of two-locus disequilibrium were not able to pick up an association, either when compared with neutrality expectations or by examining the frequency of two-locus genotypes in the four general categories (double heterozygotes, double homozygotes, and the two classes of single homozygotes). We should note that these general tests are relatively weak (e.g., see Hedrick and Thomson 1986; Weir 1990) and that more detailed analysis of haplotypes than we have carried out can give insight into the factors that have influenced genetic variation (e.g., see Klitz and Thomson 1987). In other words, it is not inconsistent with the importance of balancing selection at these loci to find that these general two-locus tests do not give statistical significance.

It is useful to briefly compare these two-locus results with those from the Hutterites (Morgan et al. 1980), a group in which there also appears to be significant selection operating (e.g., see Ober et al. 1992). When the same kind of conservative analysis that we used here was used on the Hutterite data from Morgan et al. (1980), only 16.3% of the haplotypes showed significant linkage disequilibrium in the Hutterite sample,

compared with 28.1% in the Havasupai sample. There were also in the Hutterites a number of haplotypes that had high positive D' values, but the frequencies of the haplotypes in this group were much lower than those in the comparable Havasupai group of haplotypes, possibly owing, in part, to the presence of more alleles in the Hutterite sample. In other words, both the Hutterites and Havasupai appear to have a distribution of D' values that is somewhat different from that expected under neutrality (see Hedrick and Thomson 1986), and this suggests the importance of some type of balancing selection, although is it difficult to separate out the influence of other factors such as genetic drift.

Compared with other population groups, the Havasupai show somewhat less polymorphism, for both the HLA-A and HLA-B loci. HLA variation has been reported for several other Arizona tribes: Navajo (Williams et al. 1981; Troup et al. 1982), Pima (Williams and McAuley 1992), and Hopi (Williams et al. 1981). All three are similar in frequency at HLA-A to the Havasupai, in that A2 is the most common antigen; but A30 is much more frequent in the Navajo and the Hopi than in the Havasupai or the Pima. As expected on the basis of other populations, more variants are seen in Havasupai at HLA-B than at HLA-A, although the difference is still less than that in other Native American populations. Allele frequencies at HLA-B show greater differences among the populations. While B48 is the most common among Havasupai, it is one of the least common in the Navajo and the Hopi. In the Pima, B35 and B48 are the most common, occurring at almost equal frequencies. Also common in the Pima is BN21, not even found in the Havasupai. In the Navajo and the Hopi, B39 is the most common.

We can quantify the genetic differences between these groups by calculating the genetic distance (Nei 1972) for these two loci (table 5). For HLA-A the genetic distances between the different tribes are relatively small, averaging .040 over all values, while for HLA-B the genetic distances are much greater. More specifically, the Havasupai and the Pima have a genetic distance from the other groups that is relatively large (average of .597 for the Havasupai and .313 for the Pima), while that between the Hopi and the Navajo is relatively small (average of .118). When we estimate genetic distance by combining both loci, the Havasupai have a slightly higher average value than do the other tribes.

Loci that have more variation are expected under neutrality theory to also have greater genetic distance between groups (Nei 1987), a trend that we see here for HLA-B as compared with HLA-A. However, HLA appears to be or to have been under some type of balancing selection (e.g., see Hedrick and Thomson 1983; Hedrick et al. 1991*a*), an opinion even shared by neutralists (e.g., see Nei and Hughes 1991). Further, it appears that intralocus recombination (e.g., see Belich et al. 1992; Watkins et al. 1992) and gene conversion (e.g., see Wheeler et al. 1990; Kuhner et al. 1991) between different loci may be important factors influencing genetic variation for HLA genes. Both balancing selection and the lack of independence of different amino acid substitutions because of intralocus recombination are not consistent with the neutrality assumptions usually employed when relationships between groups are determined with molecular data. As a result, it probably is not wise to use HLA to suggest relationships between these Native American groups or other human groups.

For example, Nei and Livshits (1989), using a large sample of proteins, blood groups, and DNA markers, found that Europeans and Asians are statistically more related than are Europeans and Africans and that these two groups are more related than are Asians and Africans. However, using HLA and immunoglobulins, they found that this statistical significance was not present and that the Europeans and Africans were the most unrelated of these groups. We do not know the exact cause of this incongruence, but it is an indication that

Table 5

Genetic Distance for HLA-A and HLA-B and the Two Loci Combined, between Havasupai, Hopi, and Navajo, and Pima

	Hopi	Navajo-1	Navajo-2	Pima
HLA-A:				
Havasupai	.010	.017	.087	.025
Hopi		.015	.104	.044
Navajo-1			.049	.029
Navajo-2				.019
HLA-B:				
Havasupai	.728	.629	.800	.230
Hopi		.137	.141	.355
Navajo-1			.076	.419
Navajo-2				.248
Combined:				
Havasupai	.256	.213	.310	.111
Hopi		.064	.121	.156
Navajo-1			.058	.134
Navajo-2				.085

NOTE.—Data for the Hopi and for Navajo-1 are from Williams et al. (1981); data for Navajo-2 are from Troup et al. (1982); and data for Pima are from Williams and McAuley (1992).

HLA data may give results different from those given by other molecular data that may satisfy the assumptions of neutrality more closely.

Family data were not available to permit direct ascertainment of haplotypes in the other cited studies of Native American populations, but Troup et al. (1982) used a method described by Mattuiz et al. (1970) to estimate haplotype frequencies in the Navajo, and Williams and McAuley (1992) used a pseudo gene-counting method in the Pimas (e.g., see Baur and Danilovs 1980). For the Navajo, the three most common haplotypes were A24 B35, A2 B40, and A2 B35, in order of descending frequency (Troup et al. 1982). Each of these haplotypes occurs at a low frequency or is not present in the Havasupai. Further, the most common haplotypes in the Havasupai were either in much lower frequency or missing in the Navajo sample. In the Pimas, the most common haplotypes were, in order, A2 B48, A2 B35, and A24 BN21. While the A2 B48 is also the most common haplotype in the Havasupai, A2 B35 is quite rare, and A24 BN21 is not present at all.

The differences between the Havasupai and the other groups raise the issue of the evolutionary forces that brought these differences about. Allele frequency differences could reflect differences in the ancestral populations from which each group derived. The Havasupai derive from the largest of 13 bands of Yuman-speaking Pai who inhabited the area from the Grand Canyon southward to Burro Creek and the Bill Williams River and westward from the Little Colorado River to the Colorado River valley (Martin 1986). The Pai are considered to be the local descendants of the archaeological Cerbat culture dating from 1300 A.D. in the area. The Hopi and the Pima languages are in different branches of the Uto-Aztecan language family. The Navajo are Athabascan speakers and are considered to be the most recent arrivals to the Southwest. Of all the Pai groups, the potential for admixture historically was most restricted for the Havasupai. To the north, the Grand Canyon provided an effective barrier from the Utes and the Yavapai, and to the south were longstanding enemies of the Havasupai. The Hopi were disinclined to intermarry with the Havasupai. Nevertheless, about 20% of all unions appear to have been exogamous, largely with the neighboring Hualapai, with whom the Havasupai share a common language (Martin 1986). In our study, we excluded from the analysis any Havasupais known to have mixed ancestry.

Genetic drift is also likely to have played a role in shaping the genetic differences between these groups. Historically, the population size of the Havasupai has been smaller than those the other three tribes. Visitors to the area in the 1700s reported the Havasupai regional band to number 300–350 (Coues 1900). At the beginning of the reservation period, in 1897, the Havasupai census showed 263 members. A series of epidemics further reduced the Havasupai population, to 166 by 1905. During the next generation, only 42 males and 43 females reproduced, giving an even smaller effective population size. This relative bottleneck could have contributed both to the reduction in variation for HLA, compared with that in the other tribes, and to the different allele and haplotype frequencies. This, of course, would not effect genotypic proportions—i.e., heterozygote excess—in the present generation.

Although we have been able to eliminate, to our satisfaction, the nonselective factors of different male and female frequencies and avoidance of consanguinity as the cause of our observed homozygous deficiencies, there are a number of potential selective factors that may cause a homozygote deficiency that have been suggested as important at HLA (or MHC) loci. For example, selection acting as heterozygote advantage, linked lethals, maternal/fetal interaction, negative assortative mating, and resistance to pathogens have all been suggested (e.g., see Hedrick 1990). As an example, evidence has recently been presented suggesting that, for MHC loci, homozygote deficiencies in the mouse that are similar to those documented here in the Havasupai may be due to negative assortative mating (Egid and Brown 1989; Potts et al. 1991; also see Hedrick 1992). In recent years, further data supporting the relationship of HLA variation and resistance to diseases caused by various pathogens has accumulated (e.g., see de Vries et al. 1979; Hill et al. 1991), a relationship emphasized, in a recent perspective by Black (1992), as being of great significance in Native Americans. Although the evidence presented here indicates that there has been some type of strong balancing selection in the Havasupai, we presently are unable to discriminate among these selective explanations.

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